REMARKS

Claims 1-3, 5-7, and 9-11 are all the claims pending in the application. Claims 4 and 8 have been canceled, and claim 1 has been amended. Support for the claim amendment can be found throughout the specification and originally filed claims, especially at least at Example 1 and page 3, fifth paragraph, of the specification.

Specifically, (i) the phrase "bone marrow mesenchymal cells" in step (a) is changed to "bone arnarrow mesenchymal stem cells" (*See* Example 1), (ii) the term "to" in step (b) is changed to "before" (*See* Example 1. 1. (1) and (2)), (iii) the phrase "of step (b) without carrier" is incorporated in step (c) (*See* Example 1. 2. (2)), and (iv) the phrase "in the presence of an inducer of cartilage differentiation" is further incorporated in step (c) (*See* page 3, fifth paragraph, of the specification).

Accordingly, no new matter has been introduced by these amendment to claim 1.

Present Claims Define Allowable Subject Matter

1. Claims 1-3, 5, 6 and 9-11 remain rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Goodwin *et al.* (U.S. Patent No. 5,496,722; "Goodwin #1"), Baker *et al.* (In Vitro. Cell Dcv. Biol., vol 33, page 358, 1997; "Goodwin #2"), Goodwin *et al.* (In Vitro. Cell Dev. Biol., vol 33, page 366, 1997; "Goodwin #3") and Schwarz *et al.* (USP 5,026,650; "Schwarz") in view of Unsworth *et al.* (Nature Medicine; "Unsworth"), Wikipedia, Bock *et al.* (Tissue Engineering of Cartilage and Bone; "Bock") and Bartlett (Ovarian Cancer Methods and Protocols).

a) In response to Applicants' previous argument regarding mesenchymal stem cells, the Examiner asserts that this argument is not commensurate with the scope of the claims

because "mesenchymal stem cells" is a far broader limitation than "bone marrow mesenchymal cells" recited in present claims.

For purposes of clarification, the claims have been amended to recite "bone marrow mesenchymal stem cells". Applicants, submit that Applicants prior arguments are commensurate with the scope of the claims as amended.

Further, the Examiner states, "nowhere in the previous Office Actions did the Examiner use the phrase" "mesenchymal stem cell," page 4 or otherwise." However, at page 4 of the previous Office Action of November 24, 2008, the Examiner stated, "Goodwin#1 does indeed meet these limitations in the previous action by producing cartilage tissue expressing Type II collagen from the bone marrow mesenchymal cells." (emphasis added). As discussed above, "bone marrow mesenchymal cells" is one type of "mesenchymal stem cell," and Applicants are correct in stating, "[t]he Office previously stated that Goodwin #1 discloses promoting differentiation of mesenchymal stem cells to form cartilage tissue by producing cartilage tissue expressing Type II collagen from bone marrow mesenchymal cells." Page 5, *Response to the Advisory Action, July 24, 2009.*

b) In response to Applicants' previous argument regarding the novelty of culturing mesenchymal stem cells to confluence in 2D prior to differentiation in 3D, the Examiner asserts that Applicants' cited references, Majumdar et al., Metzger et al., White et al. and Alhadlaq et al., do not explicitly discourage the culturing of mesenchymal stem cells to confluence. Further, the Examiner refers to three additional references, Kadner, Banfi and Ito, and asserts that the cited references teach confluent culture of mesenchymal stem cells.

Initially, Applicants respectfully point out that it is not required that the state of art discourages or teaches away from the confluent culture of mesenchymal stem cells. Instead,

Applicants submit that Applicants sufficiently showed that the state of art discloses or suggests the non-confluent culture of mesenchymal stem cells prior to differentiation, instead of the confluent culture as claimed.

In addition, none of the cited references teaches or suggests confluent culture of mesenchymal stem cells. Specifically, Banfi describes differentiation of bone marrow stromal cells (BMSC), which is a mixture of several different types of cells. That is, when bone marrow cells are cultured under certain conditions, stromal cell layers are produced in the form of connected cells. This stromal cell layer is made up of so-called bone marrow stromal cells, which layer comprises a variety of different cells such as fibroblast cells, mesenchymal cells, adipocytes, endothelial cells, and macrophages. Stromal cells have the capacity of supporting hematopoietic progenitor cells and make the proliferation and differentiation of hematopoietic progenitor cells possible for a period of several weeks in the absence of cytokines. During this long term of culture, an *in vitro* hematopoietic microenvironment model is generated, which model has a cell-cell interaction capacity and a capacity of secreting connective factors and cytokines. These elements enable the strict control of production of blood cells, differentiation of progenitor cells, and reproduction of stem cells. Thus, bone marrow mesenchymal stem cells are contained in the bone marrow stromal cells. In the present invention, however, only bone marrow mesenchymal stem cells are used for production of cartilage tissue under conditions as recited in claim 1. Moreover, Banfi is silent regarding any further differentiation of its cells into a cartilage tissue.

Further, another reference cited by the Examiner, Kadner (European Journal of Cardio-thoracic Surgery, 21, 1055-1060, 2002), teaches only cardiovascular differentiation of mesenchymal stem cells and does not teach or suggest confluent culture of mesenchymal stem

cells prior to chondrogenic differentiation. *See* Abstract. Moreover, the Ito reference discloses only "high-density culture" with its magnetic nanoparticles and is silent regarding any confluent culture of mesenchymal stem cells. *See* Page 119, right column.

Accordingly, in view of the state of art teaching only non-confluent culture of mesenchymal stem cells prior to differentiation, Applicants respectfully submit that one of ordinary skill in the art would have no reasonable expectation of success in differentiating mesenchymal stem cells to form cartilage tissue by using a confluent 2D culture prior to differentiation.

c) In response to Applicants' previous argument regarding the limitation of subculturing the confluent 2D cells to a 3D culture as a single element, the Examiner asserts that the recited step does not exclude additional steps such as reseeding the 2-D culture on a 3-D scaffold.

However, the Examiner appears to misunderstand Applicants' argument again because Applicants never argued that all reseeding of mesenchymal stem cells from 2D culture to 3D culture must be excluded. Rather, Applicants submit that the reseeding of mesenchymal stem cells from non-confluent 2D to 3D are not included because mesenchymal stem cells cultured to confluency have different characteristics from mesenchymal stem cells that have never been cultured to confluency.

Further, the Examiner states, "claim 1 step (b) is quite broad and as interpreted by the Examiner is not limited to a single element. The active step of "subculturing" is broad and reads on all the common steps one of ordinary skill in the art would typically take during culturing, such as seeding cells, trypsin digestion, passing cells, changing media etc." The Examiner then concludes, "claim 1 step (b) by using the active term "subculturing" does not limit the method to

a single element." Here, the Examiner appears to misunderstand the meaning of "a single element." While it may be true that the meaning of "subculturing" is broad, the step of subculturing is still a single element because it requires only one defined step, rather than multiple steps. Moreover, Applicants also point out that one of ordinary skill in the art would understand that the term, "subculturing" does not mean "changing media" at least.

d) In response to Applicants' previous argument regarding the unexpectedly superior results of not requiring a carrier, the Examiner states that if this is the inventive step then such an important exclusion should be reflected in the claims.

In response, Applicants have amended claim 1 to recite that a carrier is not required. In the present invention, differentiation of bone marrow mesenchymal stem cells to cartilage cells and formation of cartilage tissue from cartilage cells are carried out concurrently in the same vessel in the absence of a carrier, while in Goodwin, cartilage tissue is formed from differentiated cartilage cells in the presence of microcarrier beads. In Goodwin, cells are attached to microcarrier beads, and the microcarrier beads appear to function as a scaffold for differentiated cells.

For the reasons presented above, Applicants respectfully request that the above obviousness rejection be reconsidered and withdrawn.

2. Claims 1-3, 5-7 and 9-11 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over Goodwin #1, #2, #3 and Schwarz, and further in view of Yan *et al.* (US 2002/0168763; "Yan") and Simpson *et al.* (US 2002/0090725; "Simpson"). The Examiner relies on Goodwin #1, #2, #3 and Schwarz to reject Claims 1-3, 5, 6 and 9-11 as described above and further rejects claim 7 in view of Yan and Schwarz. The Examiner's detailed position is repeated at pages 11-12 of the Office Action.

The Examiner admits that Goodwin #1 does not teach the addition of dexamethasone (DEX) to their culture media. Rather, the Examiner relies on Yan for the teaching of the addition of dexamethasone.

Previously, at page 8 of Applicants' Response filed April 24, 2009 and at page 8 of Applicant's Response filed July 24, 2009, Applicants explained that while Yan adds DEX in medium to differentiate pluripotent HS stem cells into a hepatic cell line, DEX is absent in the medium to promote the mesodermal differentiation into bone marrow and cartilage. *See* Yan at [307], [310], and [330]. *Response to the Office Action, April 24, 2009 and Response to the Advisory Action, July 24, 2009.* However, again, the Examiner fails to address this argument.

Nonetheless, solely to expedite prosecution, Applicants have further amended claim 1 to recite "culturing the cells … in the presence of an inducer of cartilage differentiation."

None of the cited references teaches or suggests the combination of RWV and TGF-β as recited in present claim 1. The presence of an inducer of cartilage differentiation is effective for promoting the differentiation of the stem cells into cartilage cells. When compared with static culture in the presence of TGF-β, the combination of RWV and TGF-β makes it possible to form a large mass of cartilage tissue (*See* Example 1, Fig. 4). This suggests the importance of the combination of RWV and TGP- β. Although both Simpson and Yan disclose many culture conditions (including apparatus and means) and thus many combinations of such conditions, it would not have been obvious to find the conditions as recited in amended claims because the objectives and means are different from each other.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

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